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HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEW

Apolipoprotein E Polymorphism and Cardiovascular Disease: A HuGE Review

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This review examines the association between the apolipoprotein (apo) ε gene polymorphism (or its protein product (apo E)), metabolic regulation of cholesterol, and cardiovascular disease. The apo ε gene is located at chromosome 19q13.2. Among the variants of this gene, alleles *ε2, *ε3, and *ε4 constitute the common polymorphism found in most populations. Of these variants, apo *ε3 is the most frequent (>60%) in all populations studied. The polymorphism has functional effects on lipoprotein metabolism mediated through the hepatic binding, uptake, and catabolism of chylomicrons, chylomicron remnants, very low density lipoprotein (VLDL), and high density lipoprotein subspecies. Apo E is the primary ligand for two receptors, the low density lipoprotein (LDL) receptor (also known as the B/E receptor) found on the liver and other tissues and an apo E-specific receptor found on the liver. The coordinate interaction of these lipoprotein complexes with their receptors forms the basis for the metabolic regulation of cholesterol. Allelic variation in apo ε is consistently associated with plasma concentrations of total cholesterol, LDL cholesterol, and apo B (the major protein of LDL, VLDL, and chylomicrons). Apo ε has been studied in disorders associated with elevated cholesterol levels or lipid derangements (i.e., hyperlipoproteinemia type III, coronary heart disease, strokes, peripheral artery disease, and diabetes mellitus). The apo ε genotype yields poor predictive values when screening for clinically defined atherosclerosis despite positive, but modest associations with plaque and coronary heart disease outcomes. In addition to genotype-phenotype associations with vascular disease, the alleles and isoforms of apo ε have been related to dementias, most commonly Alzheimer's disease. *Am J Epidemiol* 2002;155:487–95.

apolipoproteins E; cardiovascular diseases; epidemiology; genetics

GENE

Apolipoprotein (apo) ε is a member of the apolipoprotein gene family. Other members of this multigene family include apo A-I, apo A-II, apo A-IV, apo C-I, apo C-II, and apo C-III. The coding regions of these genes are composed of tandem repeats of 11 codons, which suggests that they have evolved through duplications of a primordial gene (1).

The apo ε gene is located at chromosome 19q13.2 and is closely linked to the apo C-I/C-II gene complex (2). It consists of four exons and three introns spanning 3,597 nucleotides and produces a 299 amino acid polypeptide (2, 3). It is synthesized primarily in the liver, but other organs and tissues also synthesize apo E, including brain, spleen, kidneys, gonads, adrenals, and macrophages (4).

GENE VARIANTS

The structural gene is polymorphic with three common alleles, *ε2, *ε3, and *ε4, producing three isoforms of the protein, E2, E3, and E4. These isoforms differ in amino acid sequence at positions 112 and 158. Apo E3 contains cysteine at 112 and arginine at 158. Apo E2 has cysteine at both positions, and E4 has arginine at both sites (5). The apo ε gene polymorphism also has a strong effect on the level of its gene product; *ε2 is associated with higher concentrations of apo E and *ε4 with lower concentrations (6, 7).

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Abbreviations: apo, apolipoprotein; CHD, coronary heart disease; CI, confidence interval; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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While there are rare variants, it is the polymorphism with its three alleles, *ε2, *ε3, and *ε4, that has been studied in relation to cardiovascular disease. From these alleles arise six phenotypes; their ranking from most to least common is generally 3/3, 4/3, 3/2, 4/4, 4/2, and 2/2 (8). Table 1 provides gene frequencies for 11 populations, including a number of European Caucasian populations that demonstrate a geographic cline (6, 9–18). Northern Europeans (Finns, Germans) tend to have higher frequencies (~14–19 percent) of the *ε4 allele than southern Europeans (French, Italians) (~7–12 percent). Nigerians, Japanese, and Finns have relatively low frequencies (~3–4 percent) of *ε2. Mexican Americans and American Indians also have low frequencies (~2–4 percent) of the *ε2 allele. In one group consisting of nine tribes of South-American Indians (n =95), no instance of *ε2 was reported (19). Kataoka et al. speculate that the presence of *ε2 in American Indians may be the result of admixture (11). Table 2 provides genotype frequencies for these same 11 populations (6, 9–18). References for tables 1 and 2 were selected from nondiseased population cohorts to provide gene and phenotype frequencies based on large and diverse populations for international and ethnic comparisons.

FUNCTION

Plasma lipoproteins are spherical bodies composed of a nonpolar lipid core, primarily triglycerides and cholesteryl esters, with an external layer of phospholipids and apolipoproteins (20). Apolipoproteins, the only protein component of lipoproteins, combine with free cholesterol, phospholipids, cholesterol esters, and some triacylglycerols to form lipoproteins. Human plasma contains about a dozen different apolipoproteins represented by five main types (A, B, C, D, and E), some of which are further categorized into subtypes (e.g., A-I, -II, and -IV; and C-I, -II, and -III) (7). Apo E, similar to other apolipoproteins, helps to stabilize and solubolize lipoproteins as they circulate in the blood. In general, the role of apolipoproteins in lipid metabolism includes maintaining the structural integrity of lipoproteins, serving as cofactors in enzymatic reactions, and acting as ligands for lipoprotein receptors. Apo E is critical in the formation of very low density lipoprotein (VLDL) and chylomicrons.

The various apo E isoforms interact differently with specific lipoprotein receptors, ultimately altering circulating levels of cholesterol. Apo E from VLDL, chylomicrons, and chylomicron remnants binds to specific receptor cells in the liver. Carriers of the *ε2 allele are less efficient at making and transferring VLDLs and chylomicrons from the blood plasma to the liver because of its binding properties. By contrast, carriers of the *ε3 and *ε4 alleles are much more efficient in these processes. While apo E4 and E3 bind with approximately equal affinity to lipoprotein receptors, apo E2 binds with less than 2 percent of this strength (7). Thus, compared with carriers of the *ε3 or *ε4 allele, carriers of the *ε2 allele are slower to clear dietary fat from their blood (21). The difference in uptake of postprandial lipoprotein particles results in differences in regulating hepatic low density lipoprotein (LDL) receptors, which in turn contributes to genotypic differences in total and LDL cholesterol levels (6, 8, 11, 12, 22, 23).

High levels of LDL cholesterol have been associated with increased risk of coronary heart disease (CHD). Sing and Davignon demonstrated that 8.3 percent of the total variance for LDL cholesterol is accounted for by the apo ε gene locus (24). However, subsequent studies estimated variances of as low as 1.0 percent (12). Apo ε contributes more to normal cholesterol variability than any other gene identified thus far in cholesterol metabolism (24).

TABLE 1. Relative frequencies of the most common alleles for the gene locus coding for apolipoprotein ε

Population studied (reference no.)	Description of study population	Sample size	Relative frequencies		
			*ε2	*ε3	*ε4
Africans (Nigerians) (9)	Unknown	176	0.028	0.662	0.310
African Americans (10)	Population based (Alabama, Illinois, Minnesota, California)	1,612 men and women	0.131	0.668	0.201
American Indians (11)	Community based (Arizona, Oklahoma, South Dakota, North Dakota)	1,838 men	0.017	0.850	0.133
		2,703 women	0.016	0.858	0.126
Caucasians					
Framingham, Massachusetts (12)	Community based (Massachusetts)	1,123 men	0.083	0.785	0.131
		1,135 women	0.077	0.789	0.133
Munster, West Germany (13)	Factory workers	1,557 men and women	0.082	0.782	0.136
Finland (14)	Randomly selected youths (5 areas)	1,577	0.039	0.767	0.194
France (15)	Randomly selected from regional populations	504	0.081	0.802	0.117
Italy (16)	Randomly selected residents of Trieste	260	0.073	0.827	0.100
Chinese (17)	Workers from Taiyuan	141 men	0.074	0.844	0.082
Japanese (18)	General population	576	0.037	0.846	0.117
Mexican Americans (6)	Community based (Texas)	963 men and women	0.039	0.859	0.102

TABLE 2. Frequencies of apolipoprotein E phenotypes in the 11 populations referred to in table 1

Population studied (reference no.)	Description of study population	Sample size	Phenotype frequencies (%)						
			E2E2	E3E2	E3E3	E4E2	E4E3	E4E4	Variant
Africans (Nigerians) (9)	Unknown	176	0* (0)†	5* (3)†	81* (46)†	5* (3)†	66* (37)†	19* (11)†	
African Americans (10)	Population based (Alabama, Illinois, Minnesota, California)	696 men 916 women	14* (2)* 12* (1)*	124* (18)* 161* (18)*	302* (43)* 413* (45)	40* (6)* 46* (5)*	196* (28)* 242* (26)*	20* (3)* 42* (5)*	
American Indians (11)	Community based (Arizona, Oklahoma, South Dakota, North Dakota)	1,838 men 2,703 women	0† (0)* 3† (0.1)*	54† (3)* 69† (2.6)*	1,316† (71.6)* 1,978† (73.2)*	9† (0.5)* 13† (0.5)*	438† (23.9)* 613† (22.7)*	21† (1.2)* 27† (1)*	
Caucasians									
Framingham, Massachusetts (12)	Community based (Massachusetts)	1,123 men 1,135 women	10* (0.9)* 4* (0.3)*	145* (12.9)* 151* (13.3)*	707* (62.9)* 711* (62.6)*	22* (1.9)* 16* (1.4)*	205* (18.3)* 219* (19.3)*	34* (3)* 34* (3)*	
Munster, West Germany (13)	Factory workers	1,557 men and women	14† (0.9)*	183† (11.7)*	969† (62.2)*	46† (2.9)*	310† (19.9)*	35† (2.2)*	
Finland (14)	Randomly selected youths (5 regions)	1,577	5* (0.3)*	85* (5.4)*	926* (58.7)*	28* (1.8)*	483* (30.6)*	50* (3.2)*	
France (15)	Randomly selected from regional populations	504	4* (0.8)†	66* (13.09)†	324* (64.28)†	8* (1.6)†	94* (18.65)	8* (1.58)†	
Italy (16)	Randomly selected residents of Trieste	260	1‡ (0.4)‡	31‡ (12)‡	178‡ (68.4)‡	43‡ (16.5)‡	4‡ (1.5)‡	3‡ (1.2)‡	
Chinese (17)	Workers from Taiyuan	141 men	2* (1.4)*	17* (12.1)*	100* (70.9)*	0* (0)*	21* (14.9)*	1* (0.7)*	
Japanese (18)	General population	576	2* (0.3)*	35* (6.1)*	414* (71.9)*	4* (0.7)*	111* (19.3)*	10* (1.7)*	
Mexican Americans (6)	Community based (Texas)	964 men and women	2* (0.21)†	65* (6.74)†	711* (73.8)†	7* (0.73)†	167* (17.32)	11* (1.1)†	1* (0.1)†

* Frequencies and/or percentages given in the original article.
† Frequencies or percentages calculated from the percentages or frequencies given in the original article.
‡ Frequencies and percentages calculated from allele frequencies given in the original article.

DISEASES

Cardiovascular disease claims the lives of about 1 million people annually in the United States, accounting for approximately 1 in every 2.4 deaths (25). Roughly 22 percent of the country's residents have some form of cardiovascular disease, which includes CHD, stroke, arrhythmias, diseases of the arteries, including peripheral artery disease, bacterial endocarditis, cardiomyopathy, congenital heart defects, congestive heart failure, rheumatic heart disease, and valvular heart disease. Women have lower odds for developing cardiovascular disease (1 in 10) before age 60 years than men do, but their risk increases significantly after the protective effect of estrogen is lost as they pass through the climateric. It has been estimated that about half of all deaths in developed countries are caused by cardiovascular disease (26).

CHD, which accounts for 1 of every 4.7 deaths in the United States (25), has been associated with behavioral, genetic, and environmental risk factors in epidemiologic investigations. In 1981, Hopkins and Williams published a list of 246 factors associated with CHD (27). The primary risk factors linked with CHD, according to the American Heart Association, are cigarette smoking, elevated total and LDL cholesterol levels, low high density lipoprotein cholesterol level, hypertension, sedentary lifestyle, obesity, and diabetes mellitus (28). Other arterial diseases, such as thrombotic stroke and peripheral artery disease, are associated with these risk factors, although the degree of impact varies by disease. The cardinal risk factor for stroke is hypertension, although others have also been associated positively (25). Two important risk factors for peripheral artery disease are smoking and diabetes (29). Apo ε is not considered a major risk factor for any of these vascular disorders.

Epidemiologic studies have investigated the direct impact of apo ε on CHD, as well as its impact on cholesterol levels. These studies are distinguished by their focus: 1) the apo ε polymorphism as an independent risk factor for disease and 2) its contribution to cholesterol and lipoprotein levels. One study, addressing the contribution of apo ε to CHD, reported that ~6 percent of the variation in risk for CHD in North America can be attributed to this locus (30). Another study of middle-aged men from nine populations estimated a ~40 percent increased risk for CHD mortality for *ε4 carriers compared with *ε3*ε3 genotype or *ε2 carriers (31). Some studies have also suggested that *ε4 carriers are particularly prone to developing disseminated coronary lesions or to have an increased risk of death from CHD (32–35). It has been proposed that the biochemical mechanism is related to dysfunction of the E4 isoform in lipoprotein metabolism and an increased concentration of serum cholesterol and triglycerides (8, 36, 37). Studies from Finland, Scotland, and northern Ireland have shown that populations with higher cholesterol levels and higher CHD mortality rates also have a higher frequency of the *ε4 allele (31, 38). Other studies have also associated the *ε2 allele with increased CHD risk (32).

An association between apo *ε2/2 and type III hyperlipoproteinemia has been known for decades (39). This disorder is characterized by increased cholesterol and triglyceride levels, the presence of β-VLDL (cholesterol-enriched remnants of intestinal chylomicrons and hepatic VLDL), xanthomas, and premature vascular disease, both CHD and peripheral artery disease (40). Overt hyperlipoproteinemia III occurs with a frequency of 1–5 per 5,000, whereas homozygosity for *ε2/2 occurs with a frequency of 0.5–1.0 per 100 in Caucasian populations (8, 40). Thus, this genotype contributes to the hyperlipoproteinemia III phenotype without being its sole cause.

Strains of apo ε-deficient and apo ε-overexpressing transgenic mice have been developed to increase our understanding of apo ε in disease processes. Apo ε-deficient mice accumulate VLDL and remnant particles in plasma and develop atherosclerosis, even on low-fat diets (41). Increased expression of human apo *ε3 in transgenic mice results in hypertriglyceridemia (42).

In addition to being studied in association with cardiovascular disease outcomes and intermediate phenotypes, the apo ε polymorphism has been investigated as a risk factor for other chronic diseases, such as diabetes mellitus, β thalassemia, rheumatoid arthritis, Alzheimer's disease, Parkinson's disease, schizophrenia, and psychosis (43–51).

ASSOCIATIONS

There is a wealth of literature on the apo E polymorphism and attempts to associate this locus with numerous phenotypes; most of it is related to cardiovascular disease or cardiovascular disease risk factors. The citations that follow were selected to give a balanced, although not exhaustive view of genotype-phenotype studies.

Apo ε has been one of the most thoroughly studied genetic polymorphisms, particularly for its effects on lipid profiles and CHD risk. In comparisons made to determine risk, the homozygous *ε3/3 genotype is used as the referent. In general, *ε2 lowers total cholesterol levels and *ε4 raises them. The *ε2 cholesterol-lowering effect is 2–3 times that of the *ε4 cholesterol-raising effect. On average, *ε2 lowers cholesterol levels by ~14 mg/dl and *ε4 raises them by ~8 mg/dl (22). This effect has also been reported in children (52) and is evident in most populations, despite highly variable mean concentrations of cholesterol (22). The gene products of apo ε seem to function in a relatively uniform physiologic way in all populations, despite differences in genetic backgrounds, diet, and exercise patterns (22).

Various studies of vessel pathology have been conducted by using postmortem specimens, angiographic findings, and ultrasound measurements of intima-media thickness. In one autopsy study of young (aged 15–34 years) Caucasian and African-American males, the apo ε genotype accounted for 5.7 percent of the observed variation in lesions of the thoracic aorta in Caucasians and 5.9 percent in African Americans and for 5.9 percent of the variation in lesions of the abdominal aorta in Caucasians and 7.0 percent in African Americans (53). Adjustment for cholesterol levels did not appreciably change these apo ε genotypic effects. In a study of the right and left anterior descending coronary arteries and aortae from 700 male autopsy cases (Helsinki Sudden Death Study) ranging in age from 33 to 70 years, Ilveskoski et al. concluded that apo *ε4 is a significant genetic risk factor for

coronary atherosclerosis in early middle age but that it loses its importance with age (54). A small, positive association between carotid intima-media thickness, measured by ultrasound, and the *ε4 versus *ε3 allele has been documented for asymptomatic, nondiabetic patients (16). In contrast, the apo *ε3/2 genotype was associated with carotid artery atherosclerotic disease, after the contribution of established risk variables was considered in the Atherosclerosis Risk in Communities (ARIC) study (55). This association possibly was attributed to the delayed clearance of triglyceride-rich lipoproteins for *ε2 allele carriers.

Overall, clinical studies of angiography patients have failed to demonstrate conclusively a pattern of increased CHD risk for *ε4 carriers. One meta-analysis reported relative odds for men and women with clinical CHD and angiographic CHD. The overall odds ratio for CHD risk for men with the *ε4 compared with the *ε3 allele was 1.38 (95 percent confidence interval (CI): 1.22, 1.57); for women, it was 1.82 (95 percent CI: 1.30, 2.54). Relative odds for angiographic CHD were less convincing (*ε2: odds ratio = 0.76, 95 percent CI: 0.55, 1.05; *ε4: odds ratio = 1.11, 95 percent CI: 0.88, 1.40) (31). There is also some suggestion that the apo *ε2 allele may have a protective effect (8, 31); however, despite their lower cholesterol levels, *ε2 carriers are not immune from atherosclerosis.

Figure 1 shows, for Caucasian males, the apo ε gene frequencies found in three studies conducted in the United States. The Framingham Offspring Study is a community-based study of the offspring of the original Framingham cohort (aged 23–77 years) (12). The Multiple Risk Factor Intervention Trial (MRFIT) was a multicenter primary prevention trial of men aged 35–57 years at risk for CHD (32). The third study examined consecutive male coronary angiography patients (aged 32–83 years) in Oklahoma (56, 57). Only those patients with at least one vessel disease were included in the calculation of gene frequencies in the last study. Dramatic differences are not apparent across the spectrum of CHD risk.

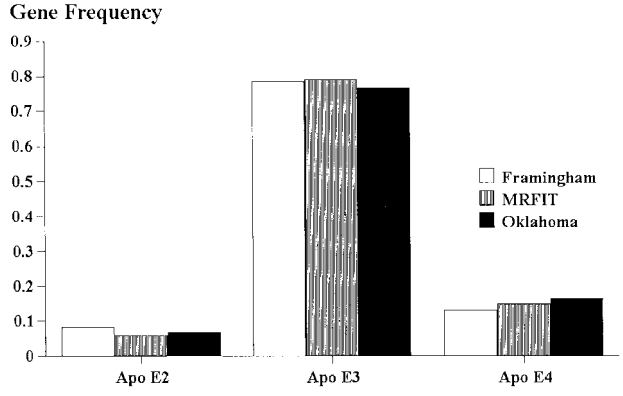

FIGURE 1. Comparison of apolipoprotein (apo) ε gene frequencies determined by protein (apo E) electrophoresis in three US studies: 1) Framingham Offspring Study, Framingham, Massachusetts—offspring of the Framingham Heart Study cohort (n = 1,123 Caucasian men; community based) (12); 2) Multiple Risk Factor Intervention Trial (MRFIT)—participants selected from the original cohort involving 22 clinical centers in the United States (n = 619 Caucasian men; primary prevention trial of at-risk middle-aged men) (32); and 3) Oklahoma Angiography Cohort, Oklahoma City, Oklahoma—coronary heart disease patients with at least one vessel disease (n = 505 Caucasian men; hospital based, 1992–1994) (author's (J. E.) data (56, 57)).

A number of studies have examined the frequency of the apo ε genotype in fatal CHD cases. The MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) Project, a multinational study sponsored by the World Health Organization, monitors trends in cardiovascular mortality and morbidity and assesses the relation of these trends to changes in risk factor levels and/or medical care. The MONICA Project suggests that an increase of 0.01 in the relative frequency of the *ε4 allele increases the CHD death rate by 24.5 per 100,000 (31). The authors of this study also suggest that the geographic distribution of apo ε alleles can be used to predict interpopulation variation in CHD mortality rates. Gerdes et al. examined the relation between apo ε genotype and a major coronary event or death in 966 Danish and Finnish survivors of myocardial infarction enrolled in the Scandinavian Simvastatin Survival Study. After evaluating 5.5 years of follow-up data on these patients, they concluded that myocardial infarction survivors carrying the *ε4 allele have an 80 percent increased risk of dying compared with other patients. They also indicated that the apo ε genotype did not predict risk of a major nonfatal coronary event (58).

Studies of centenarians show some survival advantage associated with the *ε2 allele. Altered frequencies of the apo ε polymorphism have been found in the very old compared with younger persons from the same population (59, 60). This finding may be related to both a slightly reduced risk of cardiovascular disease and a reduced risk of Alzheimer's disease. Studies that have investigated stroke risk and the apo ε polymorphism have provided mixed results. Case-control studies have reported increased frequencies of the *ε4 and *ε2 alleles among patients with ischemic cerebrovascular disease compared with controls, while other studies have shown no difference (61–63).

Apo *ε2 and *ε4 may impose additional lipid aberrations on diabetics who have elevated lipid levels and are at increased risk of CHD (43–45). One study of diabetic nephropathy has shown that the *ε2 allele is more common in patients with this complication (45). In general, there have been fewer studies of the apo ε polymorphism among stroke and diabetes patients, and the results have been less consistent than those for cholesterol variability.

Risk estimates for apo ε and Alzheimer's disease are less equivocal. A meta-analysis obtained from clinic-/autopsy-based studies provides the following summary odds for Caucasian carriers of the *ε4 allele compared with homozygous *ε3/*ε3 carriers: odds ratio for apo *ε4/2= 2.6 (95 percent CI: 1.6, 4.0); odds ratio for apo *ε4/3 = 3.2 (95 percent CI: 2.8, 3.8); and odds ratio for apo *ε4/4= 14.9 (95 percent CI: 10.8, 20.6). Summary odds for carriers of the *ε2 allele were as follows: odds ratio for apo *ε2/2 = 0.6 (95 percent CI: 0.2, 2.0) and odds ratio for apo *ε3/2 = 0.6 (95 percent CI: 0.5, 0.8) (64). The effect of

*ε4 on risk was somewhat attenuated among African Americans and Hispanics, although still present, and was accentuated among Japanese (64). Lifetime risk of developing Alzheimer's disease is 15 percent for persons with no family history of the disease. On the basis of epidemiologic data and Bayesian statistics, the risk increases to 29 percent for carriers of one *ε4 allele and is 9 percent for those with no *ε4 allele (65).

INTERACTIONS

There are numerous studies of interactions between apo ε and possible effect modifiers, such as diet, age, gender, and habits. Most important among these studies are those assessing the interaction between nutrient intake and genotype. Tikkanen et al. reported that *ε4 carriers may respond more than *ε3 and *ε2 carriers to a diet low in total fat (66), and Sarkkinen et al. showed a greater cholesterol response to changes in intake of fat and cholesterol among carriers of the *ε4 allele (67). Cobbaert et al. concluded from their study that the regional cholesterol differences in subjects from north and south Belgium, who shared a similar genetic background, could not be explained by differences in apo ε genotype distribution and serum lipoprotein(a) levels. They indicated that the less favorable *ε2 and *ε4 lipid profiles in southerners compared with northerners might reflect modulation of the apo ε gene by particular environments. They pointed out a well-documented higher intake of saturated fat and dietary cholesterol in south compared with north Belgium. On the basis of this observation, they suggested that the less favorable fat intake in southerners might explain the differences in *ε4 effects (68). A meta-analysis conducted by Ordovas et al. also proposed that the effects of apo ε genotype might be modulated via alterations of the amount and type of dietary fat (69). Other studies have shown no differential response to changes in dietary cholesterol when total fat is held constant or to total and saturated fat when cholesterol is held constant (70, 71). Boerwinkle et al. showed that, in contrast to dietary saturated fat, the apo E gene locus did not have a major effect on the response of lipid levels to increased dietary cholesterol (70). It has also been suggested that carriers of the *ε2 allele are simply less sensitive to high levels of dietary cholesterol (72). Despite conflicting evidence, there appears to be some modulation of the relation between apo ε and plasma cholesterol by fat and cholesterol intake.

In addition to evaluating diet, studies have been designed to assess interactions between apo ε and other genes, apo ε and behaviors, and apo ε and medications. Respective examples include apo ε and the angiotensin-converting enzyme insertion/deletion polymorphism and restenosis after coronary angioplasty (73), apo ε and variation in physical activity expenditure (74), and apo ε and cholesterol response to lipid-lowering drugs (75).

Gerdes et al. examined whether the beneficial effects of simvastatin treatment differed by apo ε genotype. After providing dietary advice, they randomized men and women aged 35–70 years with a history of myocardial infarction or angina, serum total cholesterol concentrations in the range of 5.5–8.0 mmol/liter, and serum triglyceride levels of less than 2.5 mmol/liter to placebo or simvastatin groups. Simvastatin treatment reduced the mortality risk more in *ε4 carriers than in other patients, although the difference was not statistically significant for the treatment by genotype interaction (58). At least two other studies have examined the influence of the apo E polymorphism on response to lipid-lowering drug treatments in patients with combined hyperlipoproteinemia and familial hypercholesterolemia (76, 77). Nestel et al. conducted a cross-over, randomized trial to examine the efficacy of simvastatin and gemfibrozil in patients with combined hyperlipoproteinemia. Efficacy was noted after 6 and 12 weeks on each treatment for the 66 subjects enrolled. The lipid-lowering responsiveness was greatest in those with the apo E2 isoform with both medications (76). Knijff et al. examined the influence of the apo E polymorphism on pretreatment plasma lipid levels and on the response to simvastatin treatment in a sample of 120 Dutch patients with heterozygous familial hypercholesterolemia. They found that the differences in pretreatment lipid levels were not related to the apo E polymorphism in these patients. With respect to the effect of 12 weeks of simvastatin treatment, a reduction of 33 percent, 38 percent, and 19 percent (on average) was found in the plasma levels of total cholesterol, LDL cholesterol, and triglycerides, respectively. Interindividual variation in response to simvastatin treatment was not related to the apo E polymorphism (77).

LABORATORY TESTS

Clinical and research laboratory tests for apo ε generally are concerned with typing the polymorphism or, less frequently, determining apo E protein concentrations in plasma or other biologic fluids. Apo E concentrations are generally higher in hypertriglyceridemia than in hypercholesterolemia and are highly variable in CHD patients and in other pathologies (7). Concentrations of apo E can be measured by radioimmunoassay, enzyme-linked immunosorbent assay, electro- or radial-immunoassay, nephelometry, or turbidimetry; however, interassay and interlaboratory comparisons are difficult because of extremely wide variation in mean values between assay formats and the lack of standardization of many of these protocols.

The apo E polymorphism was commonly screened by using phenotyping methods that detect changes in electrical charge among the protein isoforms because of sequence differences in amino acids. Apo E phenotyping is generally achieved by isoelectric focusing or two-dimensional electrophoresis. However, phenotyping is susceptible to occasional error. Post-translational changes affecting the charge of the protein are found in some pathologic conditions, for example, diabetes (78). Concentrations of apo E are usually lower in *ε4 carriers, giving faint banding patterns, and, occasionally, a rare variant has the same charge as a dominant isoform (7).

By contrast, screening for nucleotide alterations has become less prone to error. Genotyping has become relatively simple and inexpensive, making it the preferable method for

analyzing large populations. Several approaches have been taken, but all involve amplification of genomic sequences containing polymorphic sites. Amplification may entail use of allele-specific primers or a set of flanking primers, followed by endonuclease digestion, blot hybridization, single-stranded conformational polymorphism, heteroduplex analysis, or sequencing. With the advent of DNA amplification technologies, genotyping has replaced phenotyping as the standard method of determining apo ε status.

POPULATION TESTING

The sensitivity of *ε2 homozygosity in predicting type III hyperlipoproteinemia exceeds 0.90, and the presence of this genotype is a diagnostic criterion for type III disease (79). Genotype specificity is much lower, however; approximately 5 percent of homozygotes develop disease, and the positive predictive value of *ε2 homozygosity is quite low (79). Other factors, such as hypothyroidism, familial combined hyperlipoproteinemia, or diabetes, seem to be involved in the full expression of the disease. Other tests, such as the ratio between serum apo E and apo B, have been studied. This ratio is lower in type III patients than in those without disease. Marz et al. reported 95 percent sensitivity and 88 percent specificity for detecting type III hyperlipoproteinemia when a threshold value of 0.09 is used for the E/B ratio. Because of the low frequency of this disorder (1–5 per 5,000 persons) and a low positive predictive value of genotype testing, population screening is not warranted (79).

When applied to screening for CHD, the apo ε genotype is neither sensitive nor specific. The diagnostic accuracy of the four most common apo ε genotypes (3/3, 4/3, 3/2, and 4/4) from Eichner's data in patients with clinically defined CHD was assessed by using receiver operating characteristic analysis (figure 2). This study and a similar one (80), although separated geographically and temporally, yielded remarkably similar findings. The area under the receiver operating characteristic curve (a measure of how frequently the apo ε genotype distinguishes between two people, one of whom has angiographically confirmed CHD and another who does not) is 0.4914 for Eichner's data and 0.5090 for Menzel's data (80). These values indicate that the polymorphism does not distinguish clinically defined disease.

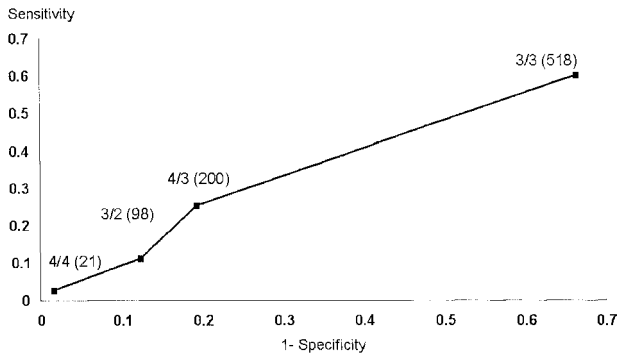

FIGURE 2. Receiver operating characteristic curve showing the usefulness of the apolipoprotein ε genotype for screening clinically defined disease. Oklahoma Angiography Cohort, Oklahoma City, Oklahoma, 1992–1994 (hospital based).

However, clinically defined disease (≥50 percent stenosis) is an imperfect measure of CHD since the presence of any arterial stenosis (<50 percent) is considered minimal or nonobstructive disease and is not normal, and the arterial lesion may be subject to hemorrhage or rupture. Lenzen et al. also investigated the association of the apo ε polymorphism with CHD (23). Their data compared survivors of myocardial infarction and healthy controls. The area under the receiver operating characteristic curve computed for the four most common genotypes from these data is 0.5097, again suggesting that the apo ε genotype is not a useful screening test for CHD (23).

In summary, apo ε was one of the first polymorphisms associated with cardiovascular disease to be studied thoroughly in both health and disease. It influences lipoprotein metabolism and the plasma concentration of total cholesterol, LDL cholesterol, apo B, and apo E and confers a risk for CHD. The American Heart Association does not mention the apo E locus as a major risk factor for CHD but does include family history as such. While apo E does help to explain interpopulation rates of CHD mortality, it does not warrant population screening as a risk factor. One exception depends on finding conclusive evidence of a genotype-drug interaction that might influence the course of disease. In such a case, screening would involve only those persons considering using the prescribed medication.

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APPENDIX. Internet Sites

Cardiovascular disease

American Heart Association:
http://www.americanheart.org
American Society for Cardiovascular Professionals/Cardiovascular Management Society:
http://www.atlanticinteractive.com/acp/acp.html
Canadian Heart and Stoke Foundation:
http://www.isfc.org
International Society and Federation of Cardiology:
http://www.isfc.org
National Health Information Center:
http://www.nih.gov
National Heart, Lung, and Blood Institute Information Center (NHLBI):
http://www.nhlbi.nih.gov/nhlbi.htm
National Organization for Rare Disorders (NORD):
http://www.nord-rdb.com/~orphan

Genetic databases

Online Mendelian Inheritance in Man (OMIM):
http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?107741#DESCRIPTION
The Genome Database (GDB):
http://www.gdb.org
GenBank:
http://www2.nebi.nlm.nih.gov